Fig. 1

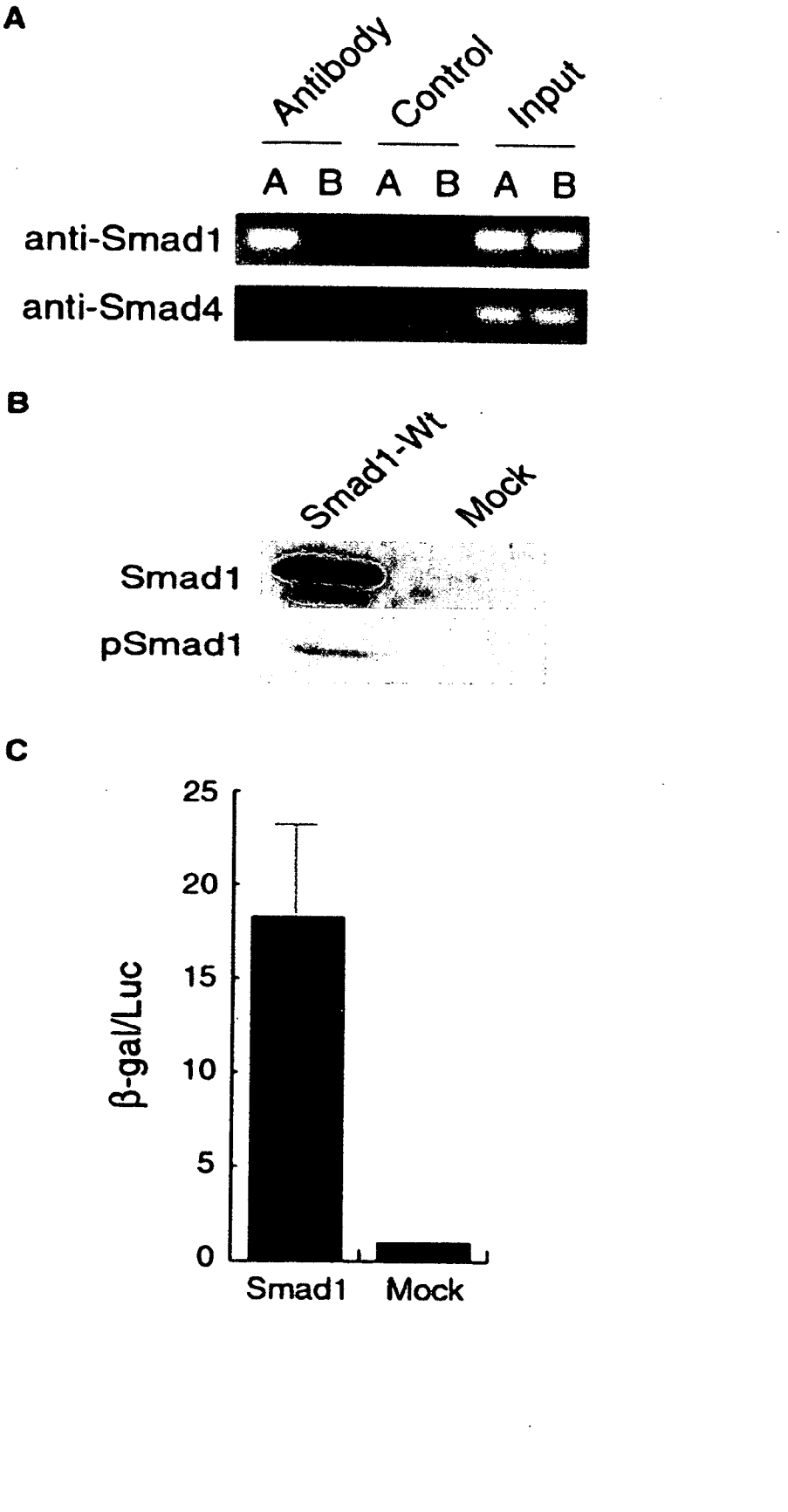

A
Antibody
Control
Input
A B A B A B
anti-Smad1
anti-Smad4
B
Smad1-Wt
Mock
Smad1
pSmad1
C
25
20
15
10
5
0
β-gal/Luc
Smad1
Mock

Fig. 2

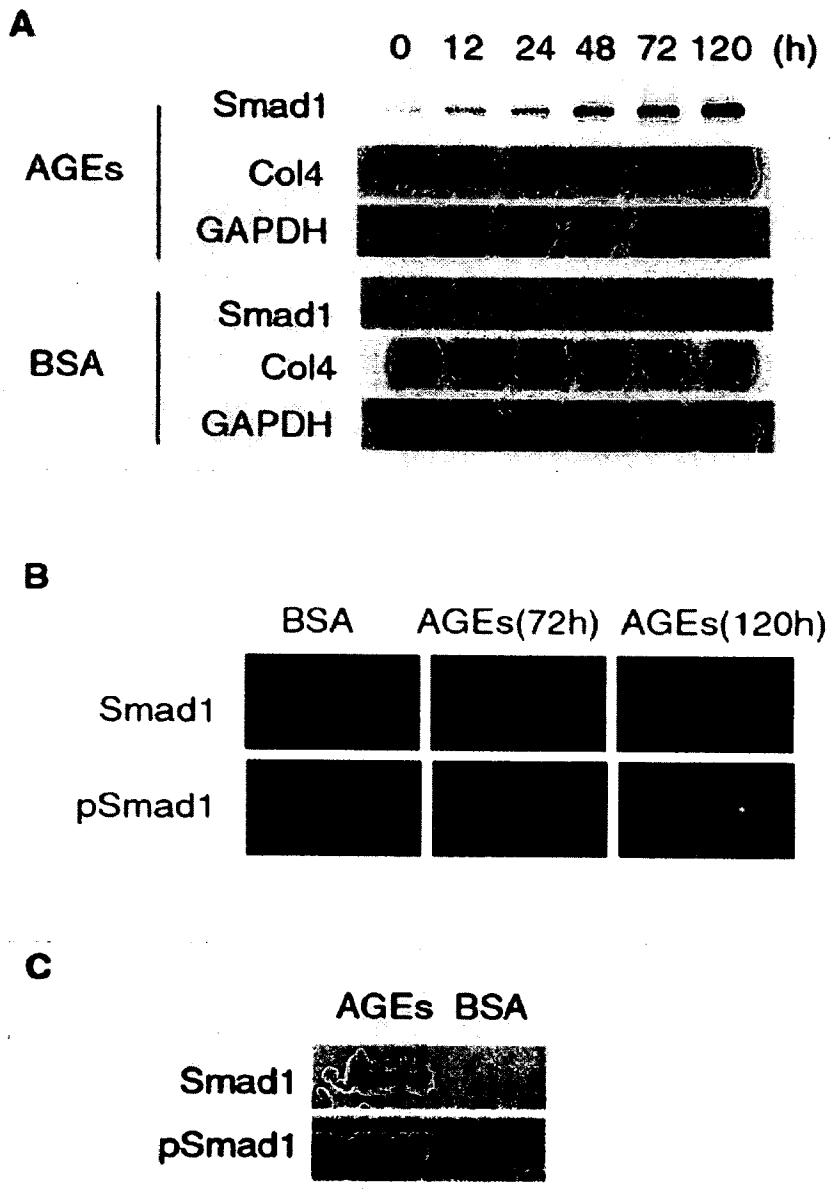
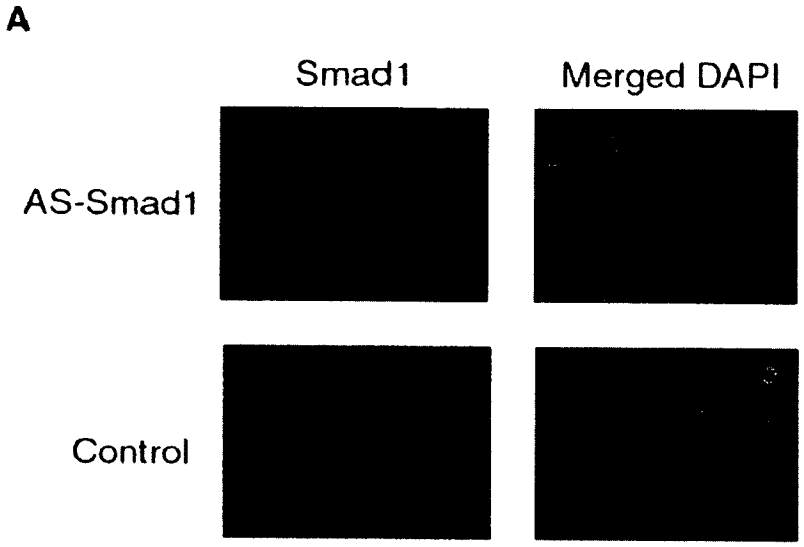

A
0 12 24 48 72 120 (h)
AGEs
Smad1
Col4
GAPDH
BSA
Smad1
Col4
GAPDH
B
BSA
AGEs(72h)
AGEs(120h)
Smad1
pSmad1
C
AGEs
BSA
Smad1
pSmad1

Fig. 3


A
Smad1
Merged DAPI
AS-Smad1
Control


B
AGE + + +
AS - + -
Control - - +
Smad1

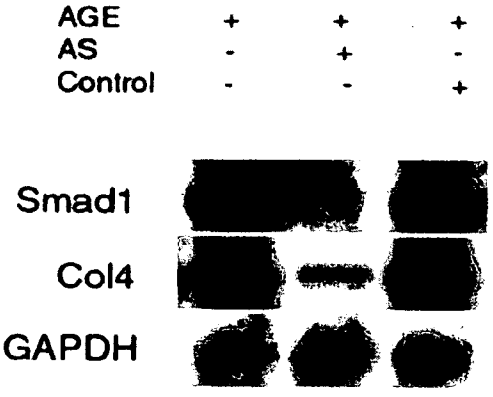

C
AGE + + +
AS - + -
Control - - +
Smad1
Col4
GAPDH

Fig. 4

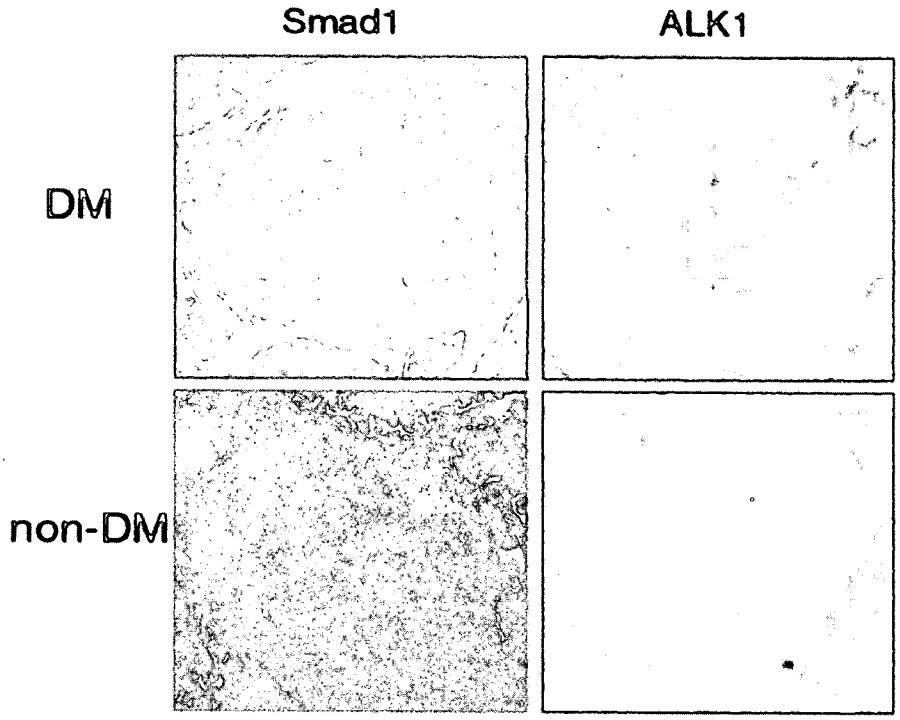


Fig. 5

Array analysis (AGEs stimulation on mMC)

	AGE/BSA Ratio	AGE/BSA(color swap)
BMP4	21.25	2.32
BMP1	2.06	2.07
SMAD1	1.27	1.22
RAGE	1.15	5.6
TGFbRII	0.49	12.1
TGFbRI	1.15	1.1
ALK3	1.18	1.3
BMPRII	2.06	4.74

Fig. 6

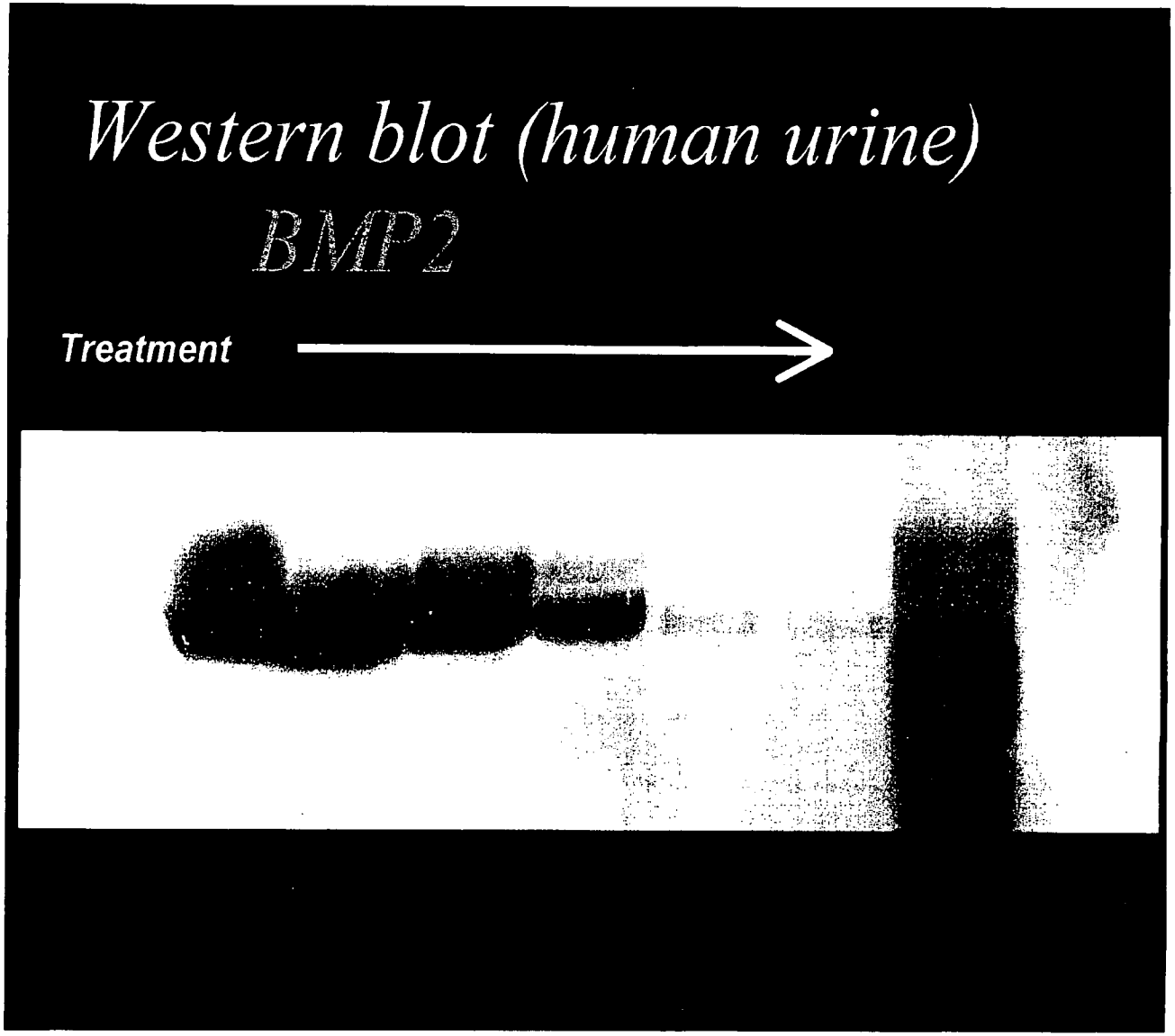


Fig. 7

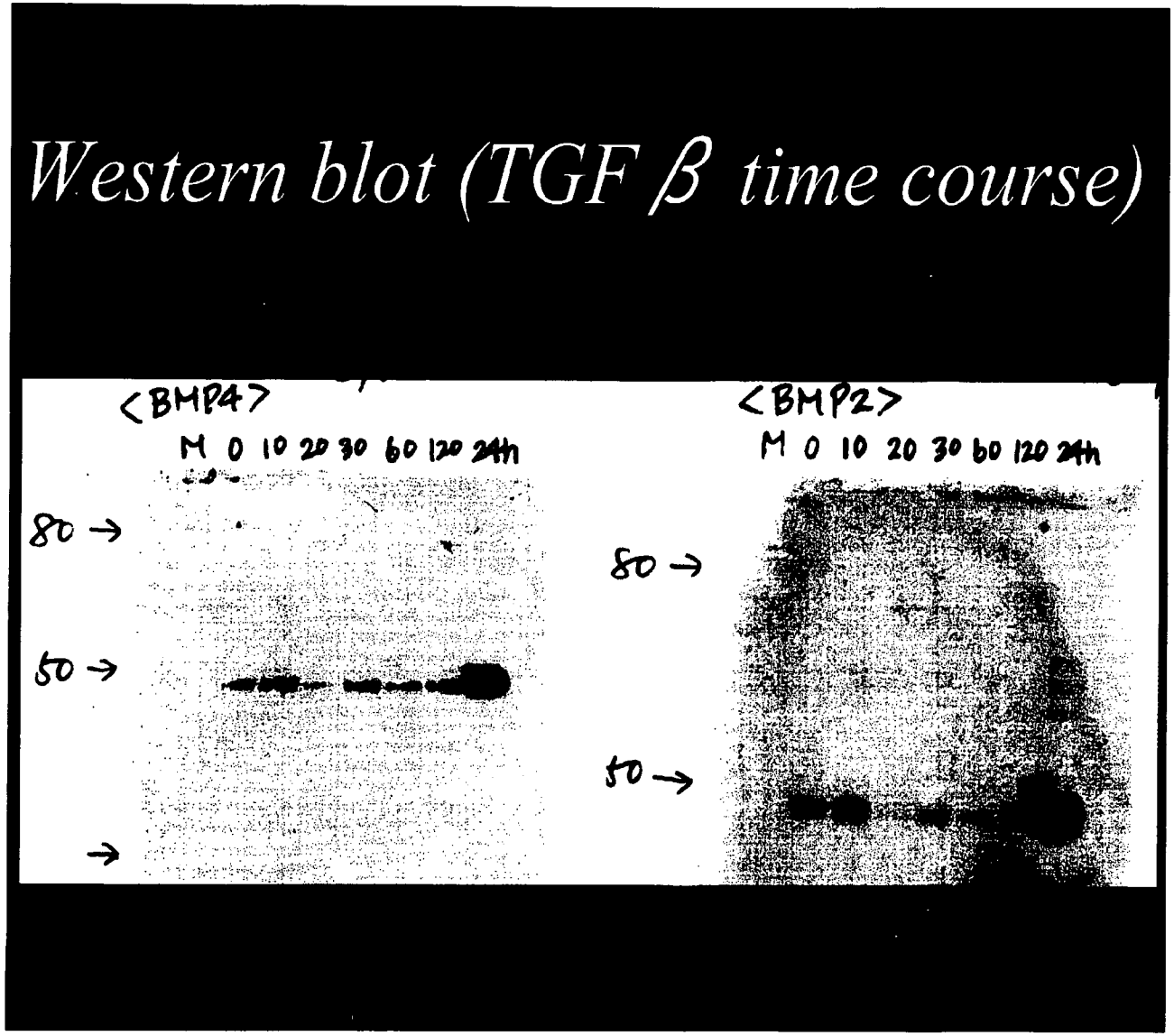

Western blot (TGF β time course)
<BMP4>
M 0 10 20 30 60 120 24h
80 →
50 →
→
<BMP2>
M 0 10 20 30 60 120 24h
80 →
50 →

Fig. 8

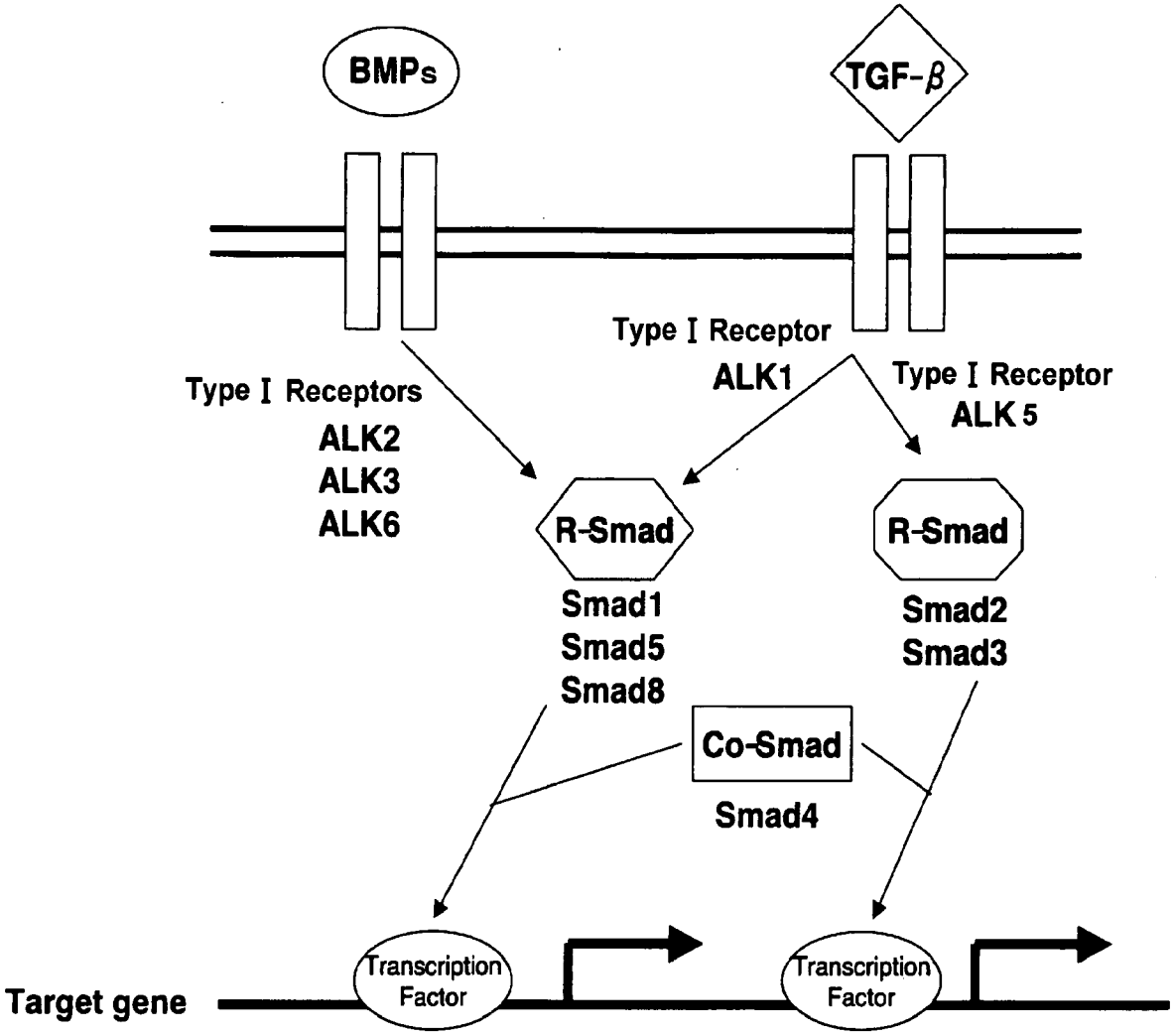

BMPs
TGF-β
Type I Receptor
ALK1
Type I Receptor
ALK 5
Type I Receptors
ALK2
ALK3
ALK6
R-Smad
R-Smad
Smad1
Smad5
Smad8
Smad2
Smad3
Co-Smad
Smad4
Transcription Factor
Transcription Factor
Target gene

Fig. 9

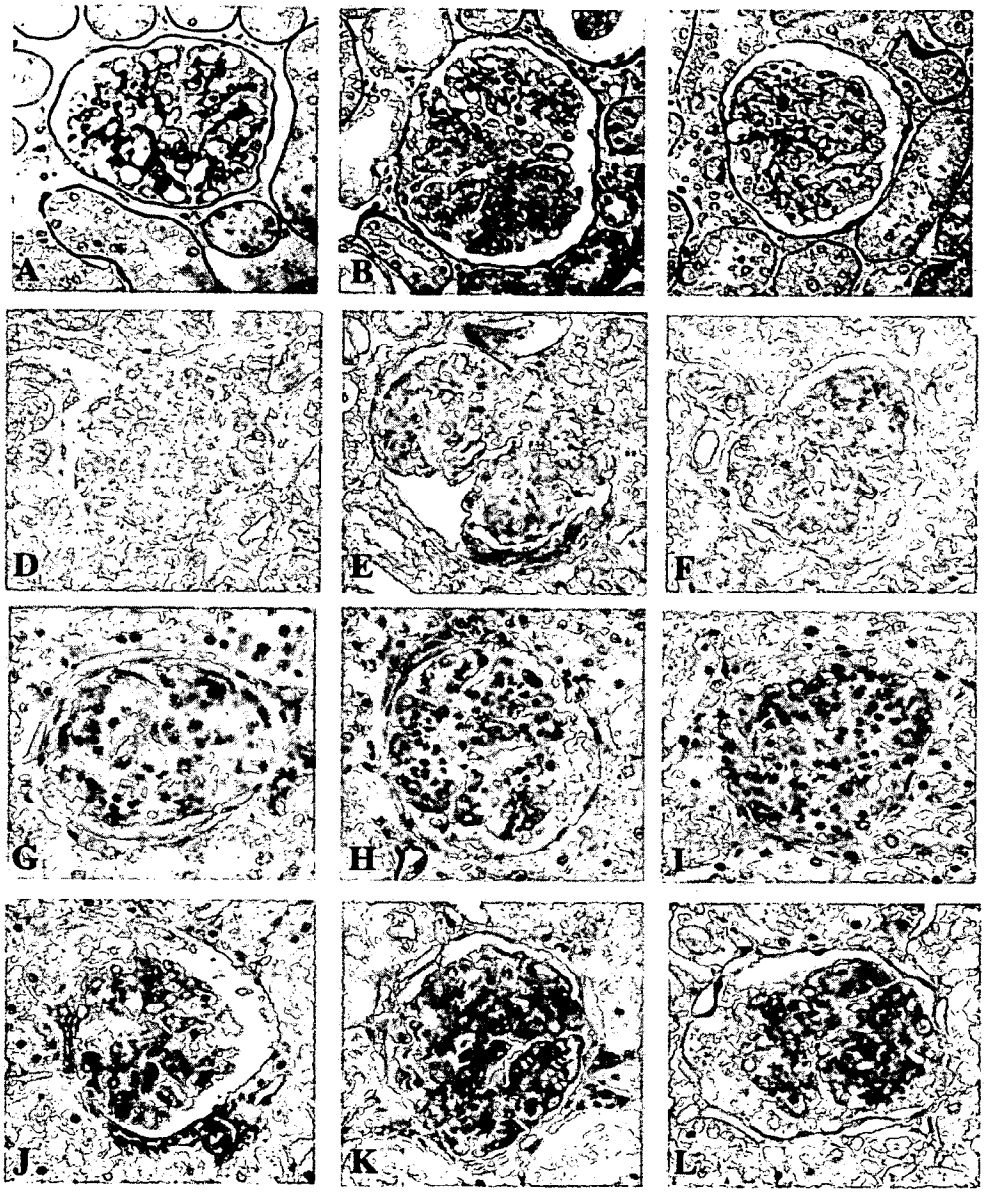


Fig. 10

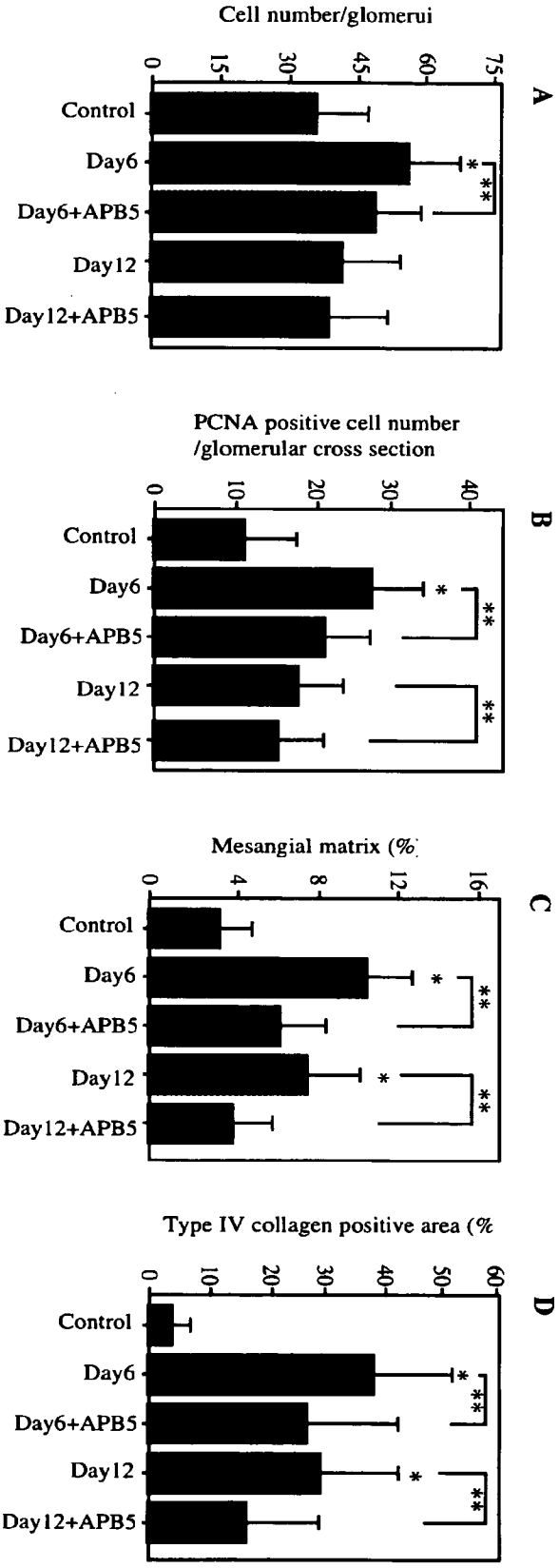

Cell number/glomerui
0
15
30
45
60
75
Control
Day6
Day6+APB5
Day12
Day12+APB5
*
**
A
PCNA positive cell number
/glomerular cross section
0
10
20
30
40
Control
Day6
Day6+APB5
Day12
Day12+APB5
*
**
**
B
Mesangial matrix (%
0
4
8
12
16
Control
Day6
Day6+APB5
Day12
Day12+APB5
*
**
*
**
C
Type IV collagen positive area (%
0
10
20
30
40
50
60
Control
Day6
Day6+APB5
Day12
Day12+APB5
*
**
*
**
D

Fig. 11

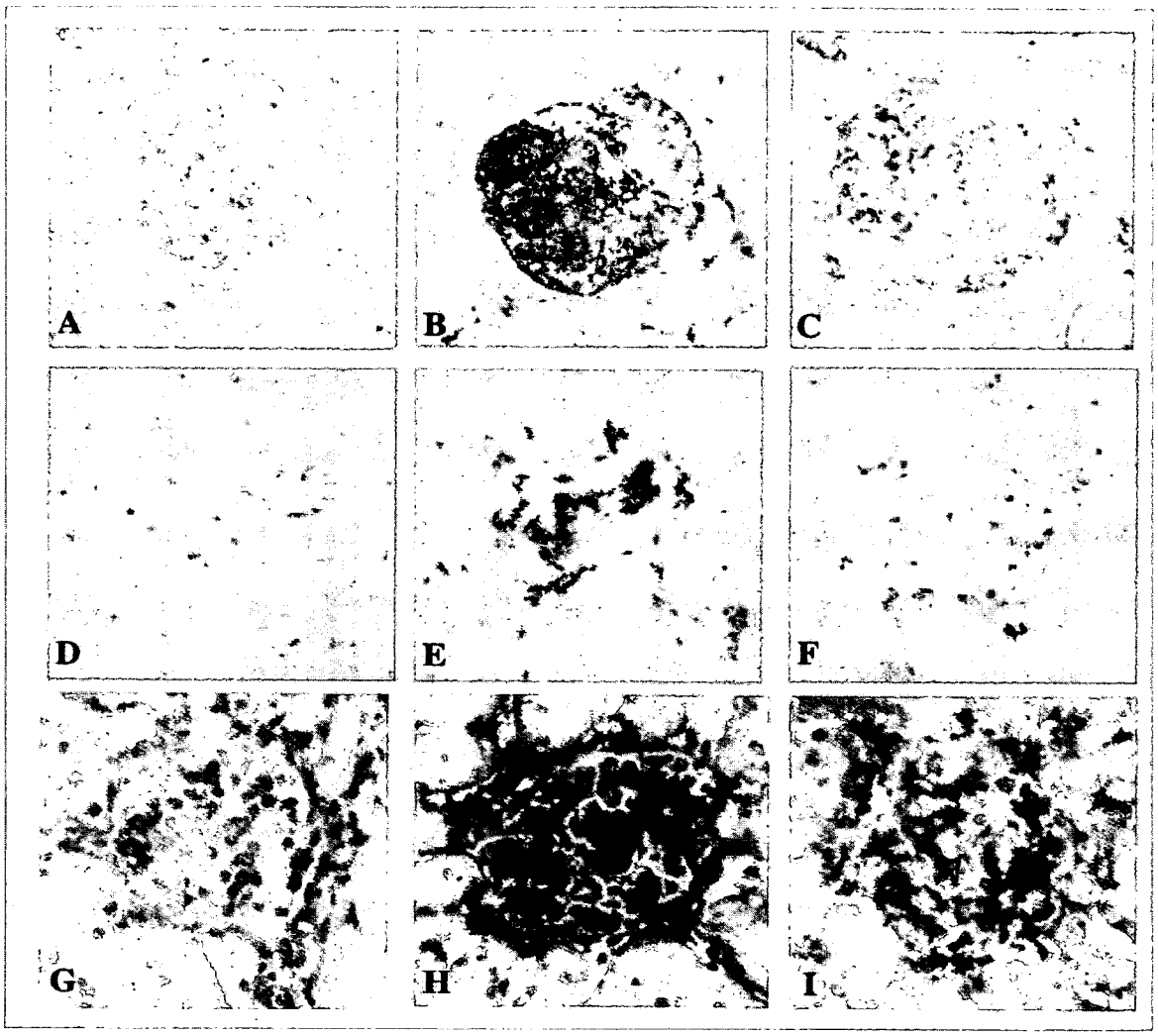


Fig. 12

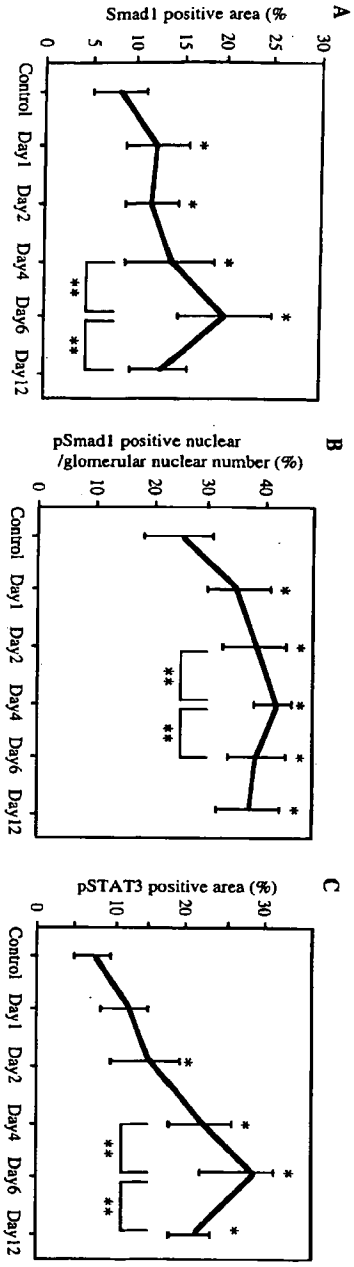

A
Smad1 positive area (%
0
5
10
15
20
25
30
Control
Day1
Day2
Day4
Day6
Day12
B
pSmad1 positive nuclear
/glomerular nuclear number (%)
0
10
20
30
40
C
pSTAT3 positive area (%)

Fig. 13

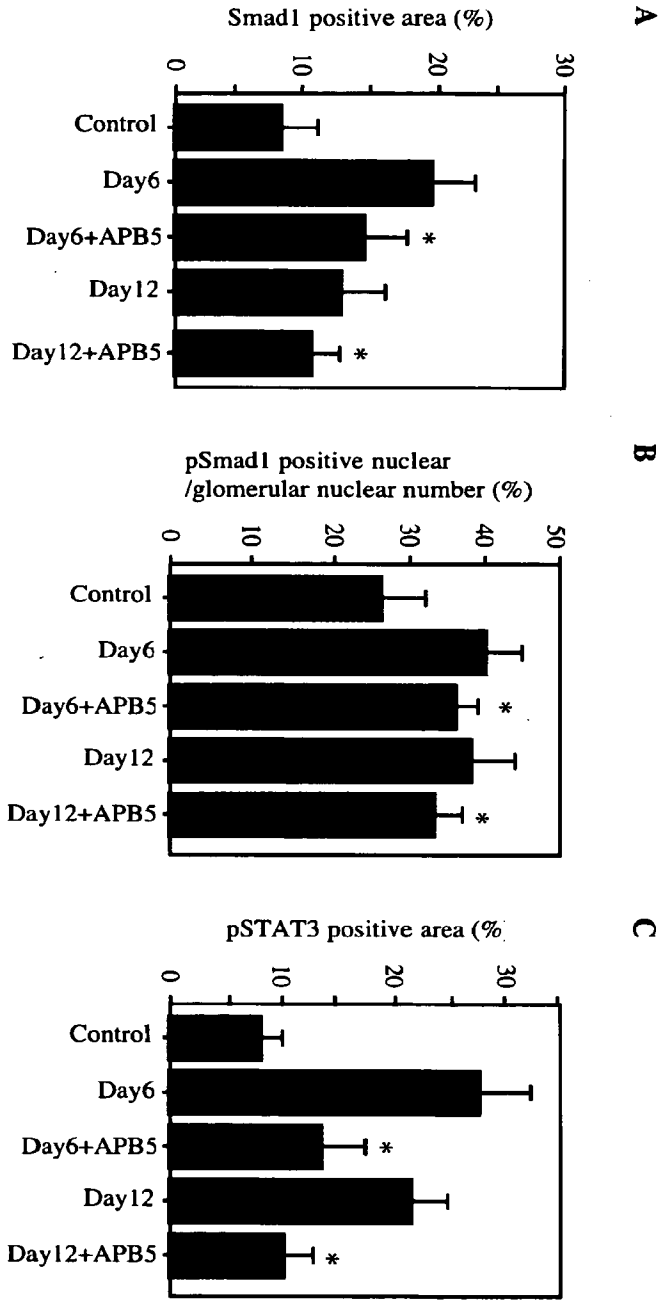

A
Smad1 positive area (%)
0
10
20
30
Control
Day6
Day6+APB5
Day12
Day12+APB5
*
*
B
pSmad1 positive nuclear
/glomerular nuclear number (%)
0
10
20
30
40
50
Control
Day6
Day6+APB5
Day12
Day12+APB5
*
*
C
pSTAT3 positive area (%
0
10
20
30
Control
Day6
Day6+APB5
Day12
Day12+APB5
*
*

Fig. 14

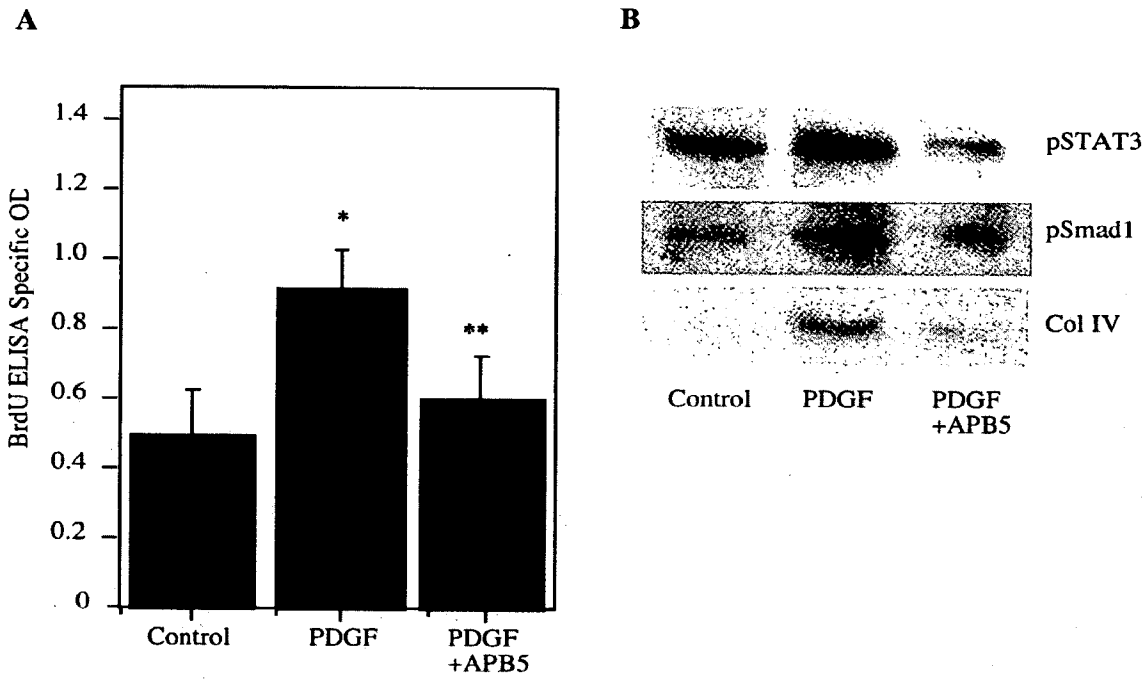

A
B
BrdU ELISA Specific OD
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
*
**
Control
PDGF
PDGF +APB5
pSTAT3
pSmad1
Col IV
Control
PDGF
PDGF +APB5

Fig. 15

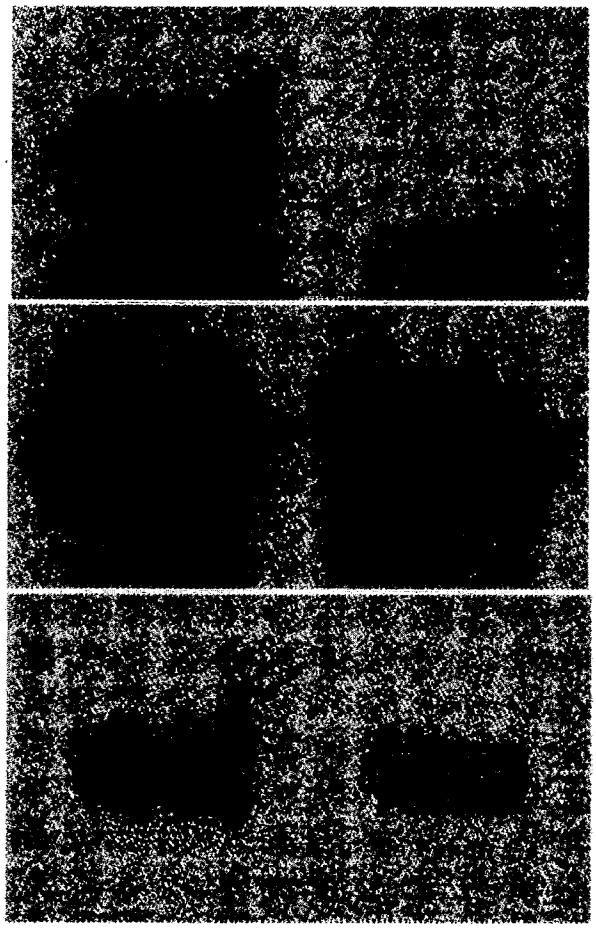


Fig. 16

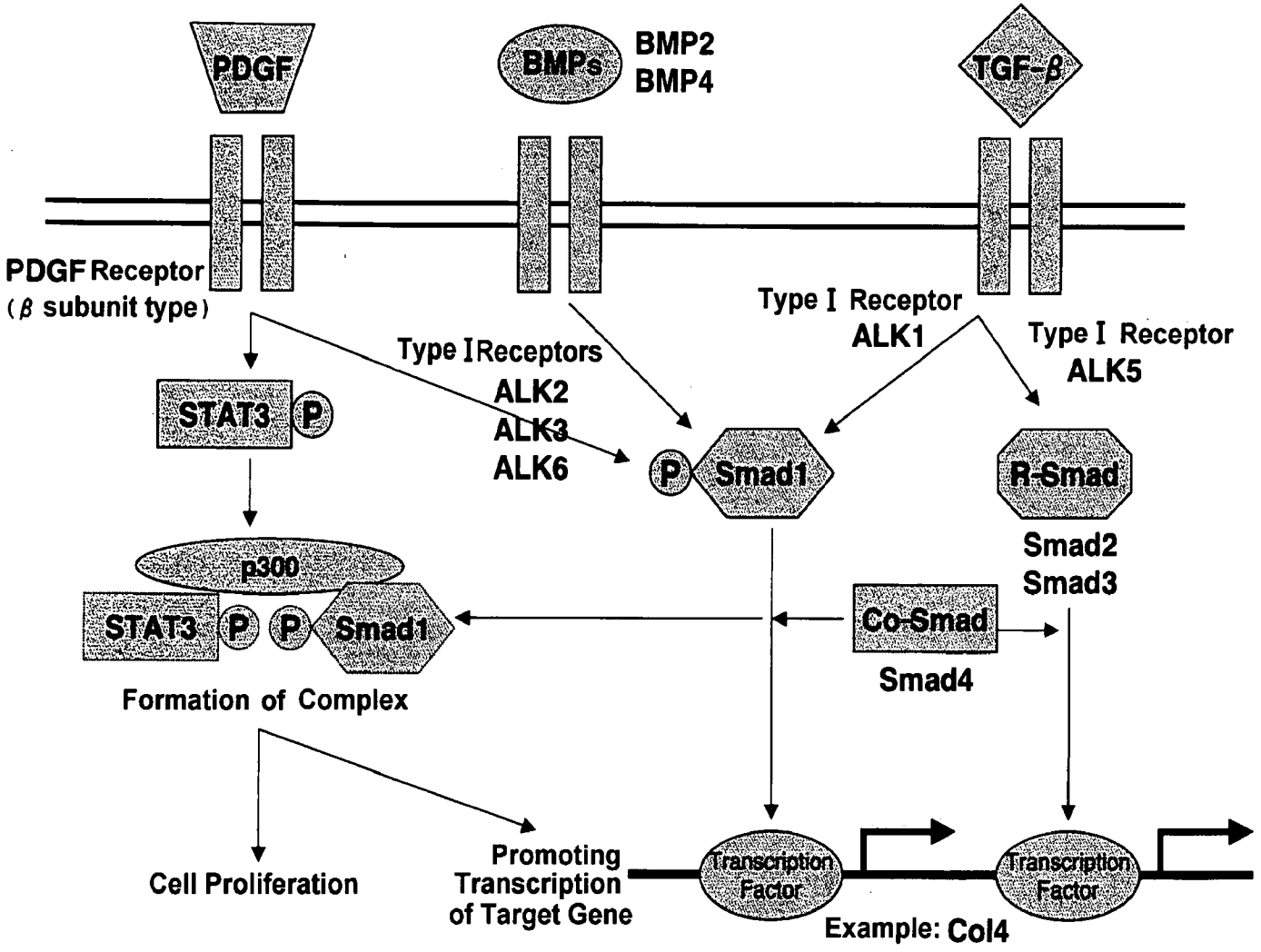
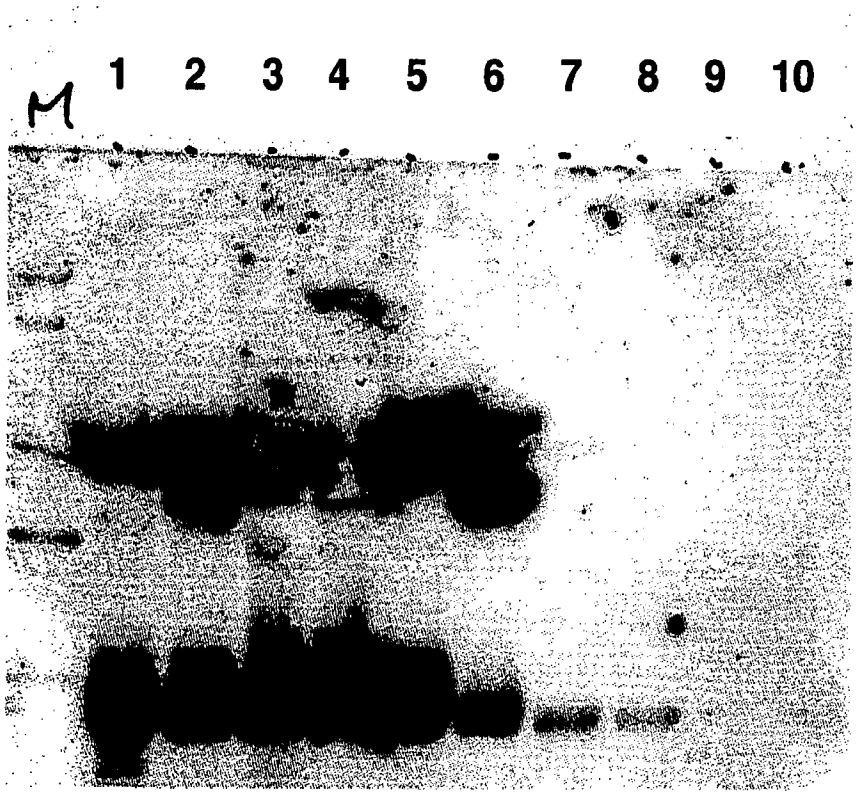

PDGF
BMPs
BMP2
BMP4
TGF-β
PDGF Receptor
(β subunit type)
Type I Receptors
ALK2
ALK3
ALK6
Type I Receptor
ALK1
Type I Receptor
ALK5
STAT3
P
P
Smad1
R-Smad
Smad2
Smad3
p300
STAT3
P
P
Smad1
Co-Smad
Smad4
Formation of Complex
Cell Proliferation
Promoting
Transcription
of Target Gene
Transcription
Factor
Transcription
Factor
Example: Col4

Fig. 17

Western blot (human urine ALK-1)



Lanes 1-5: diabetic nephropathy

Lane 6: mitochondrial disease in which diabetes is complicated with sclerosing, renal proliferative disease

Lanes 7-8: diabetes + nephritis (without sclerosis)

Lanes 9-1: normal

Fig. 18

Western blot (human urine ALK-1)

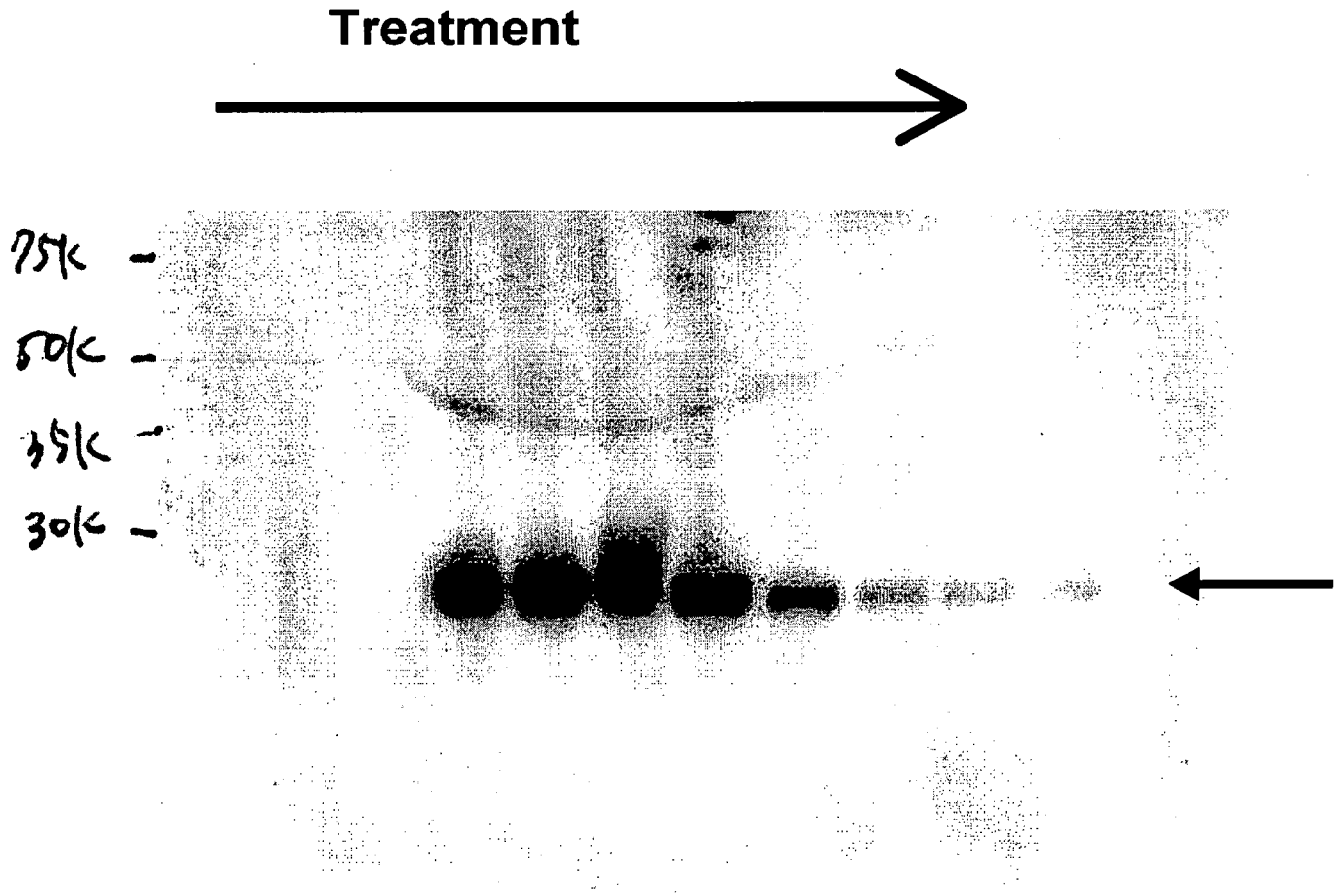
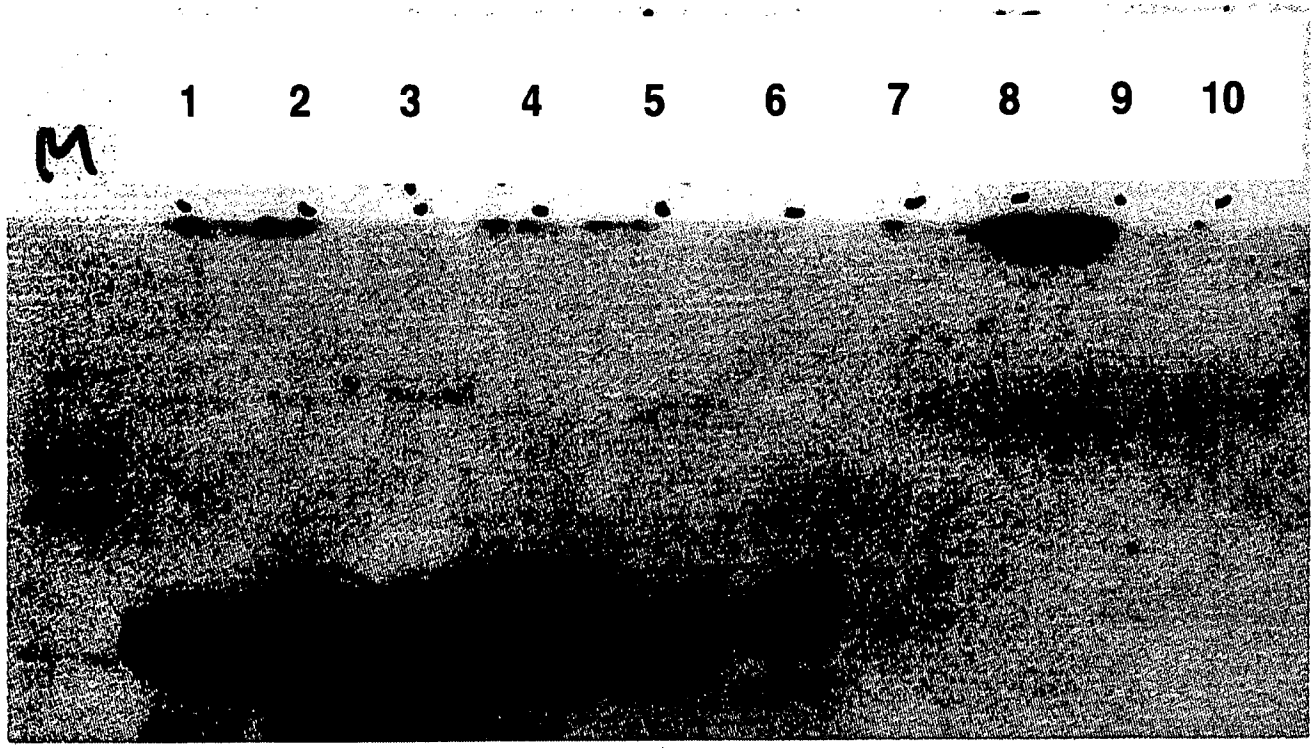


Fig. 19

Western blot (human urine Smad1)



Lanes 1-5: diabetic nephropathy

Lane 6: mitochondrial disease in which diabetes is complicated with sclerosing, renal proliferative disease

Lanes 7-8: diabetes + nephritis (without sclerosis)

Lanes 9-1: normal